Human Leukocyte Antigen HLA-B27 Detection Kit (Real-time PCR)

Instruction for Use (V1.0)

[REF] UBP-G00150H

[Specification] 50 tests/kit

[Research Use Only]

This kit is suitable for qualitative detection of the DNA in the human leukocyte antigen subtypes HLA-B*2702, HLA-B*2704 and HLA-B*2705.

This kit can be used as an aid for ankylosing spondylitis detection. Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease that mainly affects the spine and can involve the sacroiliac joints and surrounding joints to varying degrees. It has been revealed that AS exhibits obvious familial aggregation and is closely related to human leukocyte antigen HLA-B27. In humans, more than 70 HLA-B27 subtypes have been discovered and identified, and of them, HLA-B*2702, HLA-B*2704 and HLA-B*2705 are the most common subtypes related to the disease [1-10]. In China, Singapore, Japan and Taiwan district, HLA-B27 is most common and accounts for approximately 54%, followed by HLA-B*2705 which accounts for 41% [1-4]. This kit can detect the DNA in HLA-B*2702, HLA-B*2704 and HLA-B*2705, but does not differentiate them from each other.

Experimental operators should have received professional training in gene amplification or molecular biologic testing, and have qualifications relevant to experimental operation. The laboratory should be equipped with rational biological safety precautions and protective procedures.

[Test Principle]

This kit uses fluorescence-based PCR for qualitative detection of the DNA in the subtypes HLA-B*2702, HLA-B*2704 and HLA-B*2705. The HLA-B27 gene is located on the short arm of human chromosome 6. So far, more than 70 HLA-B27 subtypes have been discovered and identified, and there are differences in gene sequence among these subtypes. This kit contains primers and probes specific for HLA-B*2702, HLA-B*2704 and HLA-B*2705 in exon 3; its internal control is designed in the conserved region of the Globin gene and serves as a non-competitive internal standard that can monitor the entire experimental process. This kit contains an enzyme system composed of dUTP/UNG, which can effectively prevent environmental contamination. In the kit, the target gene detection probe is labeled with the fluorophore FAM, and the internal control gene detection probe is labeled with the fluorophore VIC.

[Kit Contents]

No.	Name	Specification	Description
1	HLA Primer probe	100 μL/vial×1	Primers/Probes for HLA-B27 and internal control testing
2	HLA PCR buffer	875 μL/vial×1	Containing nucleoside triphosphate, magnesium ion, purified water
3	HLA Taq polymerase	25 μL/vial×1	DNA polymerase and Uracil-DNA glycosylase
4	HLA Positive control	50 μL/vial×1	HLA-B*2704 positive plasmids

Note: The components of the kit in different batches are not interchangeable.

[Storage Conditions and Shelf Life]

This kit should be stored at -18°C protected from light. Its shelf life is 18 months. It should be transported using airtight foam boxes with dry ice or ice bags. After opening, the kit should be temporarily stored at 4°C protected from light for no more than 15 days (validation has not been performed for storage for > 15 days); it is recommended not to store the kit at 37°C for more than 5 days; the kit shall not be subjected to repeated freezing and thawing for more than 5 cycles (validation has not been performed for > 5 cycles), and given that the enzyme should not be subjected to repeated freezing and thawing, it is recommended that after opening, the kit be used up once for all, or the remaining product be stored at 4°C. Repeated freezing and thawing is prohibited.

[Applicable Equipment]

ABI 7500, ABI Stepone, Roche LightCycler 480, Stratagene MX 3000P Real-time Fluorescence-based qPCR Instrument, Ultrassay XP96 Real Time qPCR System.

[Acceptable Specimens]

1. Samples are collected from confirmed or suspected AS patients. Samples should be collected aseptically.
2. Collected samples are whole blood samples from confirmed or suspected AS patients. The sample volume is 0.5 mL, and the anticoagulant EDTA is added to each sample. A label with a unique identification number is affixed to the exterior of each tube. Repeated freezing and thawing of samples should be avoided during transportation and storage. If the condition -20°C cannot be ensured, samples should be transported at 0-8°C. The samples may be stored at -20°C for 6-8 months and below -70°C for a longer time. Necessary information such as the sample number, the date of disease onset, and the date of sample collection, should be attached during transportation and storage.
3. For whole blood samples stored at 4°C for 5-6 months, the detection accuracy can reach more than 95%.

[Test Procedures]

1. Sample processing

Extract and purify human genomic DNA according to the instructions for use of the corresponding whole blood genomic DNA extraction kit.

2. Preparation of reagents for amplification (PCR Area I)

Take out the primer/probe, PCR buffer and Taq enzyme from the kit, thaw them at room temperature, centrifuge at low speed, and collect the solution that may adhere to the tube wall and tube cap to the bottom of the tube. Pipette the primer/probe and Taq enzyme into the PCR buffer tube, and mix well. Add 20.0 μL of the mixture to the established fluorescence-based qPCR tube or 96-well plate, then transfer it to the sample processing area.

3. Sample loading (PCR Area II)

Add 5.0 μL of the sample prepared in Step 1 (including the positive control) into the PCR tube containing the PCR buffer.

4. RT-PCR (PCR Area III)

Put the reaction tube into the fluorescence-based PCR instrument, and set the cycle parameters as follows:

Step	No. of Cycles	Temperature	Duration	Fluorescence Signal Collection
1	1	37°C	2 mins	NO
2	1	95°C	1 min	NO
3	40	95°C	5 secs	NO
		59°C	20 secs	Yes

Fluorescence signals are collected in the FAM (HLA-B27) and VIC/HEX (internal reference) channels at 59°C.

[Reference Range]

Based on the analysis of laboratory results, the Ct (cycle threshold) value for this kit is finally established as Ct=40 by using the ROC curve.

[Explanation of Test Results]

After the end of reaction, the instrument automatically saves the results. After analyzing the image, adjust the Start value, End value and Threshold value of the Baseline [the Start value can fall within a range of 3-6 (**recommended to be adjusted to 5**), and the End value falls within a range of 15-20 (**recommended to be adjusted to 18**); when reading the Ct value, select the negative control well, and adjust the threshold line so that it is located above all noise lines and slightly above the amplification curve of the negative control well. The concentrations of the internal reference and the target gene in the positive control should be controlled within a range corresponding to a Ct value of 22-26, not requiring the same Ct values; the fluorescence signals of the internal reference gene and the target gene in the negative control should be negative, that is, the test results obtained by the instrument show no Ct value or no typical S-shaped amplification curve; the above conditions need to be met at the same time in the same test, otherwise PCR is considered invalid and a re-test is required. Interpretation is detailed below:

1. When the test sample shows no Ct value or no typical S-shaped amplification curve for the target gene (Ct value of the target gene > Ct value of the internal reference gene), and a typical S-shaped amplification curve with a Ct value for the internal reference, then the test sample is considered negative;
2. When the test sample shows a typical S-shaped amplification curve with a Ct value for both the target gene and the internal reference gene, then the test sample is considered positive;
3. When the test sample shows an abnormal amplification curve (such as an exorbitant baseline) for the target gene, then the test sample is suspected to be positive. For a suspected sample, a re-test should be performed, and if the re-test shows a Ct value and a typical S-shaped amplification curve, then the sample is determined to be a positive sample, otherwise, it is determined to be a negative sample; when a re-test is performed, a new blood sample should be taken for DNA extraction;
4. The test results obtained by this kit are for research use only. Any of unreasonable processes of sample collection, transportation and handling/processing may result in false negative results; variations in target sequences may result in false negative results.
5. Amplification diagram and analysis example for detection by the kit (see Figure 1).

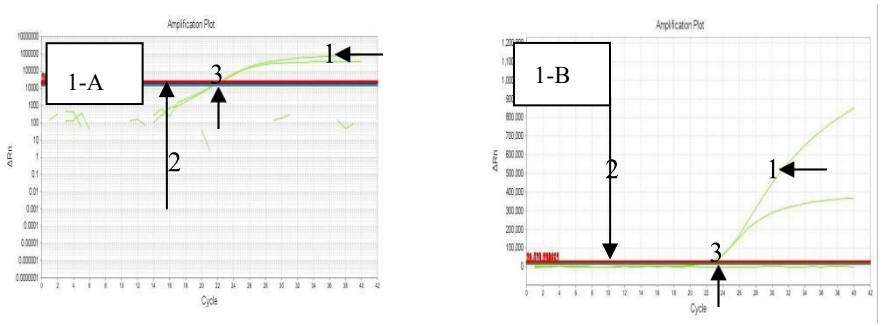

Figure 1: Amplification curve. 1-A Exponential amplification. 1-B Linear amplification: 1 - amplification curve, 2 - threshold line, 3 - Ct value.

[Limitations of Test Method]

The test results obtained by this kit are for research use only and should be interpreted in conjunction with the patient's symptoms/vital signs, medical history, other laboratory findings and outcome for comprehensive analysis. The test results are related to the quality of sample collection, processing, transportation and storage processes, and any errors in the processes will result in false negative results. If cross-contamination is not well controlled during sample processing, false positive results may occur.

[Product Performance]

1. Appearance
1.1 Appearance: The secondary packaging of the kit should be intact without damage and with complete legible printed contents including the product name, batch number, expiry date, production address and contact information.
1.2 Components: Reagents are transparent clear solutions.
2. Specificity: The negative percent agreement (NPA) for 10 negative reference standards is 10/10;
3. Accuracy: The positive percent agreement (PPA) for 8 positive reference standards is 8/8;
4. Sensitivity: The LLOD reference standard (genomic DNA concentration is about 1 ng/ul) tests completely positive;
5. Repeatability: CV ≤ 5% for the Ct value in the precision reference standard subjected to 10 replicate assays;
6. The negative control tests negative for both the internal reference gene and the target gene, i.e., the test results obtained by the instrument show no Ct value or no typical S-shaped amplification curve;
7. According to the laboratory results, the accuracy and specificity of this kit is over 99%; experiments for analysis of the sensitivity of this kit show that its LLOD can reach 0.3 ng/μL for the DNA extracted from whole blood.
8. The test results obtained by this kit will not be affected by hemoglobin < 800 g/L, bilirubin < 700 umol/L, and blood lipids (triglycerides) < 7 mmol/L in blood.

[Precautions]

1. The whole test process should be performed in three areas: Area I for reagent preparation, Area II for sample processing and reaction system preparation, and Area III for amplification, fluorescence detection and result analysis. The instruments, equipment and work uniforms in each area should be used independently to prevent contamination.
2. During operation, operators should always pay attention to avoiding DNA/DNase contamination, and use disposable gloves (frequently replaced) that contain no fluorescent substances, disposable imported thin-wall 200 μL PCR 8-tube strips (or 96-well PCR plates with optical films), and pipette tips (with self-unloading filters), and should not touch the reaction tubes directly with hands.
3. Biosafety cabinets (BSCs) should be used at the time of sample handling to ensure the safety of operators and prevent environmental contamination. Harmful and toxic samples and reagents in experiments should be properly placed and kept by designated personnel; wastes should be placed in dedicated containers for proper disposal. Instruments and equipment including operation benches, pipettes, centrifuges and PCR systems should be frequently wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Experimental rooms and ultra-clean workbenches should be treated with UV lamps regularly and after each experiment.
4. Reagents in centrifuge tubes should be fully thawed, mixed well, and centrifuged for a few seconds before use, so that the liquids are concentrated at the bottom of centrifuge tubes. When preparing a reaction system, pay attention to the following notes: all liquids should be mixed well on a vortex mixer as much as possible, without pipetting to avoid bubbles, and centrifuging at low speed should be performed for a few seconds after preparation of the reaction system. Use the kit within its shelf life. Do not mix its components in different batches.

[References]

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[12] Park KS, Kang SY, Lee WI. HLA-B27 subtypes in Korean patients with ankylosing spondylitis. Korean J Lab Med. 2008, 28(1):46-52.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
	Use By		Date of Manufacture
	Temperature Limitation		Consult Instructions for Use
LOT	Lot Number		Manufacturer
Σ	Number of Tests	REF	Reference Number
			Any warnings and/or precaution to take

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